REMARKS

Reconsideration of the allowability of the present application is requested respectfully.

Status of the Claims

Claims 1 to 11 and 21 to 30 are pending. Claims 1 to 11 and 21 to 26, 29 and 30 were acted upon by the Examiner in her Action dated April 23, 2002. Claims 27 and 28 were not acted upon by Examiner; however the Examiner indicated on page 2 of the Action that none of the claims had been allowed. No claims have been amended. No claims have been cancelled. Accordingly, Claims 1 to 11 and 21 to 30 are presented for examination.

In response to the Examiner's Action dated April 23, 2002, applicants traverse respectfully the Examiner's rejection of Claims 1 to 11 and 21 to 30.

Summary of the Rejections

The Examiner has rejected Claims 1 to 5, 7 to 10, 21, 22, 24 to 26, 29 and 30 under 35 U.S.C. §103(a) as being unpatentable over U.S. Patent No. 5,399,346 ("Anderson et al.") taken with European Patent Application No. 0381490 ("Greenberger et al.") and Boswell et al. Exp. Hematol. 11:315-323 (1983). The Examiner has rejected Claims 1 to 10, 21 to 26, 29 and 30 under 35 U.S.C. §103(a) as being unpatentable over Anderson et al., Greenberger et al., and Boswell et al., and in further view of Lozier et al. Hum. Gene Ther. 5:313-322 (1994). The Examiner has rejected additionally Claims 1 to 5 and 7 to 11 under 35 U.S.C. §103(a) as being unpatentable over Anderson et al., Greenberger et al., and Boswell et al., and further in view of Lobb et al. BBRC 178:1498-1504 (1991).

The 35 U.S.C §103(a) Rejections

The Examiner's §103(a) rejection of Claims 1 to 5, 7 to 10, 21, 22, 24 to 26, 29 and 30 as being unpatentable over Anderson et al., taken with Greenberger et al. and Boswell et al. is traversed respectfully.

Applicants submit that the Examiner has not satisfied the three basic criteria required to establish a *prima facie* case of obviousness. The MPEP §2143 states:

> "To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the publications themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or publications when combined) must teach or suggest all the claim limitations.
> The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991)."

In the arguments presented below, applicants will demonstrate that there is no basis to modify or combine the cited publications, that there was no reasonable expectation that the result of the combined disclosures of the publications would be a success, and that the combined disclosure of the publications do not teach or suggest all of the claim limitations.

Anderson et al. discloses transfection of tumor-infiltrating lymphocytes (TILs) and the cryopreservation of transfected TILs. Greenberger et al. discloses using bone marrow stromal cells (BMSCs) for gene therapy, but does not teach cryopreservation of BMSCs. Boswell et al. discloses cryopreservation of untransfected bone marrow cells. There is no evidence of record that would suggest to or motivate one skilled in the art that the disclosures of <u>any</u> of the publications be combined; in fact, there is evidence that they not be combined.

The Examiner asserts that the cryopreservation of TILs, as disclosed in Anderson et al., would not be different from cryopreservation of BMSCs and, therefore, one of skill in the art would combine Anderson et al. with the other publications. In particular, the Examiner has asserted that cryopreservation of one cell type would not be different from cryopreservation of a different cell type because "both T cells and BMSCs are derived from bone marrow". This assertion is wrong. It is well established in the art that different methods of cryopreservation are used typically for different cell types. This fact is illustrated by the experiments described in the enclosed copy of the Zaheer et al. publication (Zaheer et al., "Differential sensitivity to cryopreservation of clonogenic progenitor cells and stromal precursors from leukemic and normal bone marrow," Stem Cells 12:180-186 (1994), Exhibit 1). Zaheer et al. demonstrates that cryopreserved bone marrow cells failed to form a confluent, adherent stromal layer (i.e., the BMSCs present in the cryopreserved bone marrow were damaged). In contrast, Zaheer et al. also demonstrates that, for nonadherent bone marrow cells, proliferation and the ability to differentiate are not adversely affected by cryopreservation. Therefore, Zaheer et al. teaches that cryopreservation has a different effect on BMSCs than on other bone marrow cells, including pluripotent hematopoietic stem cells, lymphoid stem cells, and T cell progenitor cells, all of which can differentiate into TILs. Based on this teaching, a TIL cryopreservation method would not be expected to be effective for BMSCs. Therefore, one of skill in the art would not look to a method of cryopreserving TILs in order to cryopreserve transfected BMSCs. Thus, there is no suggestion/motivation to combine the cited publications as the Examiner has done.

The Examiner's assumption that TILs and BMSCs would react in the same manner to cryopreservation is in complete opposition to what is known in art regarding the cryopreservation of adherent (BMSCs) and nonadherent cells (TILs and their progenitors).

Enclosed is Exhibit 2, which consists of a copy of a technical information sheet from Mediatech, Inc. (2001), a company that specializes in providing reagents for the *in vitro* maintenance and culturing of cells; the information in this sheet is evidence that cryopreservation solutions for adherent and nonadherent cells differ. In general, a cryopreservation solution having 10% DMSO and 90% fresh medium is used for adherent cells whereas a cryopreservation solution having 10% DMSO, 45% fresh medium, and 45% spent (used) medium is used for nonadherent cells. Accordingly, the skilled artisan would not look to a TIL publication to determine the proper conditions for cryopreservation of a BMSC. Therefore, the skilled artisan would not combine the teachings of the cited publications. Accordingly, the Examiner's rejection is not valid because the criteria that a suggestion or motivation, either in the cited publications themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the teachings of the publications or to combine the publication teachings has not been met.

As noted above, a skilled artisan would not look to a TIL publication for guidance on how to cryopreserve BMSCs and a method of cryopreserving BMSCs based upon a method of cryopreserving TILs would not be expected to work. Zaheer et al. teaches that cryopreservation has a different effect on BMSCs than on other bone marrow cells. Based on this teaching, it would be unreasonable to expect a TIL cryopreservation method to be effective for BMSCs. Furthermore, since none of the cited publications discloses expression levels after cryopreservation, it would be unreasonable to expect the product of the combined disclosures of the publications would have "a level of expression of the exogenous gene which is at least about 77% of said predetermined value", as recited in Claim 1.

Thus, the Examiner's rejection is also not valid because the criteria of a reasonable expectation of success has not been met.

In order to establish a *prima facie* case of obviousness, all the claim recitations must be taught or suggested by the prior art. MPEP §2143.03. Applicants submit that the combined disclosures of Anderson et al. with Greenberger et al. and Boswell et al. do not teach or suggest all the recitations of the presently claimed invention.

Part (b) of Claim 1 states "cryopreserving the transfected BMSCs which in the thawed state have a level of expression of the exogenous gene which is at least about 77% of said predetermined value". Greenberger et al. discloses transfecting BMSCs and using them for gene therapy, but does not disclose a method of preserving BMSCs. Combining Greenberger et al. with the cryopreservation method of Boswell et al. does not teach or suggest the recitation in part (b) of Claim 1. Simply stated, none of the cited publications discloses or suggests the level of expression of an exogenous gene in a transfected cell after thawing.

Furthermore, assuming that one would be motivated to combine Anderson et al. with Greenberger et al. and Boswell et al., Anderson et al. does not teach a comparison of exogenous gene expression between thawed TILs and TILs which have not been frozen. The Examiner does not dispute this. However, on page 4 of the Office Action, the Examiner has stated that "the burden is upon the applicant to prove that the prior art products do not necessarily or inherently possess characteristics of the claimed product". Applicants assert that they have no such burden as stated in the MPEP §2112 (emphasis added):

> "The fact that a certain result or characteristic <u>may</u> occur or be present in the prior art is not sufficient to establish the inherency of that result or characteristic. *In re Rijckaert*, 9 F.3d 1531, 1534, 28 USPQ2d 1955, 1957 (Fed. Cir. 1993) (reversed rejection because inherency was based on what would result due to optimization of conditions, not what was necessarily present in the prior art); *In re Oelrich*, 666 F.2d 578, 581-82, 212 USPQ 323, 326 (CCPA 1981). "To establish inherency, the extrinsic evidence 'must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill. Inherency, however, may not be established by probabilities or

possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient.'" *In re Robertson*, 169 F.3d 743, 745, 49 USPQ2d 1949, 1950-51 (Fed. Cir. 1999) (citations omitted)...

In relying upon the theory of inherency, the examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic necessarily flows from the teachings of the applied prior art."

Just because the expression of an exogenous gene in TILs *may* be 77% when the cells are thawed is not sufficient to establish inherency. The Examiner has not provided a basis in fact and/or technical reasoning to support a position that the transfected TILs of Anderson et al. in the thawed state have a level of expression of the exogenous gene which is at least about 77% of non-frozen TILs and that this feature is *necessarily present* such that it would be recognized by those of skill in the art. Accordingly, applicants have no burden to prove the TILs in Anderson et al. do not necessarily or inherently possess a 77% expression level for an exogenous gene.

Applicants submit that the combination of the disclosures of Anderson et al. with Greenberger et al. and Boswell et al. do not suggest all the recitations of the presently claimed invention. Accordingly, the Examiner's rejection is not valid for this reason also.

Even if one were to assume that the Examiner has established a proper basis for a *prima facie* case, applicants submit that the present §103(a) rejection is based on hindsight. There are at least two lines of evidence demonstrating the nonobviousness of the claims; (1) the absence of intervening art disclosing a method of cryopreserving BMSCs such that they retain at least 77% of expression of an exogenous gene; and (2) the solution of a long felt need.

There has been an absence of intervening art disclosing a method of cryopreserving BMSCs such that they retain at least 77% of expression of an exogenous gene. Greenberger et al. was published in 1990. Despite the approximate five year period between the filing of

Greenberger et al. and the priority date of the present application, there is no indication in the publications cited by the Examiner that a BMSC was cryopreserved such that it retained at least 77% of expression of an exogenous gene. The failure to cryopreserve BMSCs retaining at least a 77% expression level is evidence of the difficulty of retaining high levels of expression in transfected BMSCs following cryopreservation. Indeed Zaheer et al., published in 1994, demonstrates the difficulty in effectively cryopreserving BMSCs such that they can grow in a monolayer, let alone express an exogenous gene at levels as high as 77%.

Given the importance of developing BMSCs useful for gene therapy, if methods of cryopreserving BMSCs retaining at least a 77% expression level were obvious, such methods would have been developed in the intervening five-year period between the date of publication (August 8, 1990) of Greenberger et al. and the priority date (December 29, 1995) of the present application. Furthermore, Zaheer et al. indicates as recently as one year before the priority date of the present application, methods of cryopreserving BMSCs were not well established. Given the absence of intervening art describing a method of cryopreserving BMSCs, applicants submit that the invention as presently claimed is nonobvious in view of Anderson et al., Greenberger et al., and Boswell et al.

There has been a long-felt need in the art for gene therapeutic methods. As stated on page 2, lines 20 to 23, of the instant application, "a major obstacle to gene therapies based on the modification of stromal cells is the procurement and sustained availability of therapeutically useful numbers of stromal cells". The presently claimed invention overcomes this obstacle by providing a method for cryopreserving BMSCs such that they retain at least 77% of expression of an exogenous gene. Given such high levels of expression of a therapeutic exogenous gene product by these BMSCs, the presently claimed invention provides therapeutically useful numbers of stromal cells.

Greenberger et al., which discloses transfection of BMSCs, was published in 1990. However, until the making of the presently claimed invention, the sustained availability of therapeutically useful numbers of BMSCs remained a problem. The combination of Anderson et al., Greenberger et al., and Boswell et al. does not teach or suggest how to overcome this problem. That the presently claimed invention satisfies a long-felt need for procurement and sustained availability of therapeutically useful numbers of BMSCs is additional evidence of the nonobviousness of the claimed invention.

In view of the above, applicants respectfully request withdrawal of the §103(a) rejection of Claims 1 to 5, 7 to 10, 21, 22, 24 to 26, 29 and 30.

As discussed below, the Lozier et al. and Lobb et al. publications provide no basis to overcome the deficiencies of Anderson et al., Greenberger et al., and Boswell et al.

The Examiner has rejected Claims 1 to 10, 21 to 26, 29 and 30 under 35 U.S.C. §103(a) as being unpatentable over Anderson et al. in view of Greenberger et al., and Boswell et al. and further in view of Lozier et al. (*Hum. Gene Ther.*).

Applicants respectfully traverse the rejection.

Lozier et al. discloses transfected BMSCs, namely canine BMSCs transfected with Factor IX. Except for the use of canine BMSCs, Lozier et al. does not disclose any relevant information beyond the information present in Greenberger et al. In fact, Lozier et al. on page 320, first full paragraph, teaches a method in which stromal cells are frozen, thawed, and then transfected. This is in contrast to the presently claimed methods in which BMSCs are transfected, then frozen and thawed such that they retain a 77% expression level of the transfected gene. Furthermore, the Examiner has stated (emphasis added) "as long as cells are viable, an exogenous gene *could be* expressed at a appropriate level meeting the claim limitation". As discussed above, "The fact that a certain result or characteristic may occur or

be present in the prior art is not sufficient to establish the inherency of that result or characteristic." MPEP §2112 citing *In re Rijckaert*, 9 F.3d 1531, 1534, 28 USPQ2d 1955, 1957 (Fed. Cir. 1993). "Inherency, however, may not be established by probabilities or possibilities." MPEP §2112 citing *In re Oelrich*, 666 F.2d 578, 581-82, 212 USPQ 323, 326 (CCPA 1981). Once again, "the examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic necessarily flows from the teachings of the applied prior art." MPEP §2112. Simply stating that "an exogenous gene *could be* expressed at a appropriate level" is not a basis in fact and furthermore does not reasonably support that a 77% expression level of an exogenous gene is an inherent characteristic that necessarily flows from Anderson et al. or Lozier et al. Thus, a *prima facie* case of obviousness has not been met.

Since Lozier et al. does not provide any information that overcomes the deficiencies in the other cited publications, applicants request respectfully withdrawal of the obviousness rejection of Claims 1 to 10 which additionally relies on Lozier et al.

Claims 1 to 5 and 7 to 11 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Anderson et al. in view of Greenberger et al., and Boswell et al., and further in view of Lobb et al. (*Biochem. Biophy. Res. Com.*). Although Claims 27 and 28 have not been acted upon by the Examiner, the argument presented below also applies to these claims.

The Examiner's §103(a) rejection of Claims 1 to 5 and 7 to 11 is traversed.

Lobb et al. discloses the expression of secreted recombinant soluble VCAM-1 (rsV-CAM-1) in CHO cells followed by purification of the secreted recombinant protein. rsVCAM-1 lacks a transmembrane region (see Figure 1 of Lobb et al.) and thus is not a cell surface molecule. In fact, Lobb et al. does not disclose expression of any cell surface

molecules. Thus, the Examiner's assertion that Lobb et al. can be "applied for the teaching of expressing cell surface molecules" is without basis. In addition, Lobb et al. does not provide any information that overcomes the deficiencies in the other publications. Accordingly, applicants respectfully request withdrawal of the §103(a) rejection of Claims 1 to 5 and 7 to 11.

Enclosed herewith in duplicate is a Petition for one-month extension of time to respond to the Examiner's Action and a Notice of Appeal. The Commissioner is hereby authorized to charge any additional fees or credit any overpayment associated with this communication to Deposit Account No. 19-5425.

Respectfully submitted,

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